

# Root development under control of magnesium availability

Yao Fang Niu<sup>1,2</sup>, Gu Lei Jin<sup>3</sup>, and Yong Song Zhang<sup>1,\*</sup>

<sup>1</sup>Ministry of Education Key Laboratory of Environmental Remediation and Ecosystem Health; College of Environmental and Resources Sciences; Zhejiang University; Hangzhou, PR China; <sup>2</sup>Department of Horticulture; College of Agronomy and Biotechnology; Zhejiang University; Hangzhou, PR China; <sup>3</sup>Institute of Bioinformatics; College of Agronomy and Biotechnology; Zhejiang University; Hangzhou, PR China

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Roots are reported to be plastic in response to nutrient supply, but relatively little is known about their development in response to magnesium (Mg) availability. Here, we showed the influence of both low and high Mg availability on the development of roots including root hairs and highlighted insights into the regulatory role of Mg availability on root hair development and its mechanism in *Arabidopsis* with combining our published research. Mg concentration in roots decreased quickly after the removal of Mg from the nutrient solution and increased progressively with increasing exogenous Mg supply in the media. However, transcriptome analysis suggested that Mg starvation did not alter the expression of most genes potentially involved in the transport. Primary root elongation and lateral root formation in *Arabidopsis* were not influenced by low Mg but inhibited by high Mg after one-week period. Moreover, low Mg availability significantly increased but high Mg reduced the initiation, density and length of root hairs, which through the characterized  $\text{Ca}^{2+}$  and ROS signal transduction pathways. More physiological mechanisms underlying Mg-regulated root development remain to be elucidated in future researches.

Magnesium (Mg), the largest hydrated radius (0.428 nm), the smallest ionic radius (0.072 nm) and the highest charge density,<sup>1</sup> is an essential element for a vast number of fundamental biochemical processes in all living cells in plants. Concentrations of Mg in soil solutions lie between 0.125 and 8.5 mM,<sup>2</sup> depending on soil texture and cation exchange capacity of the soil,<sup>3</sup> the concentration of competing cations, water availability or excessive leaching, crop cultivation and fertilizer regime.<sup>4,5</sup> However, long-term unbalanced crop fertilization practice neglecting Mg depletion of soils and cation competition and subsequent leaching lead to Mg deficiency in plants, decreased productivity and quality in agriculture practice worldwide.<sup>6</sup>

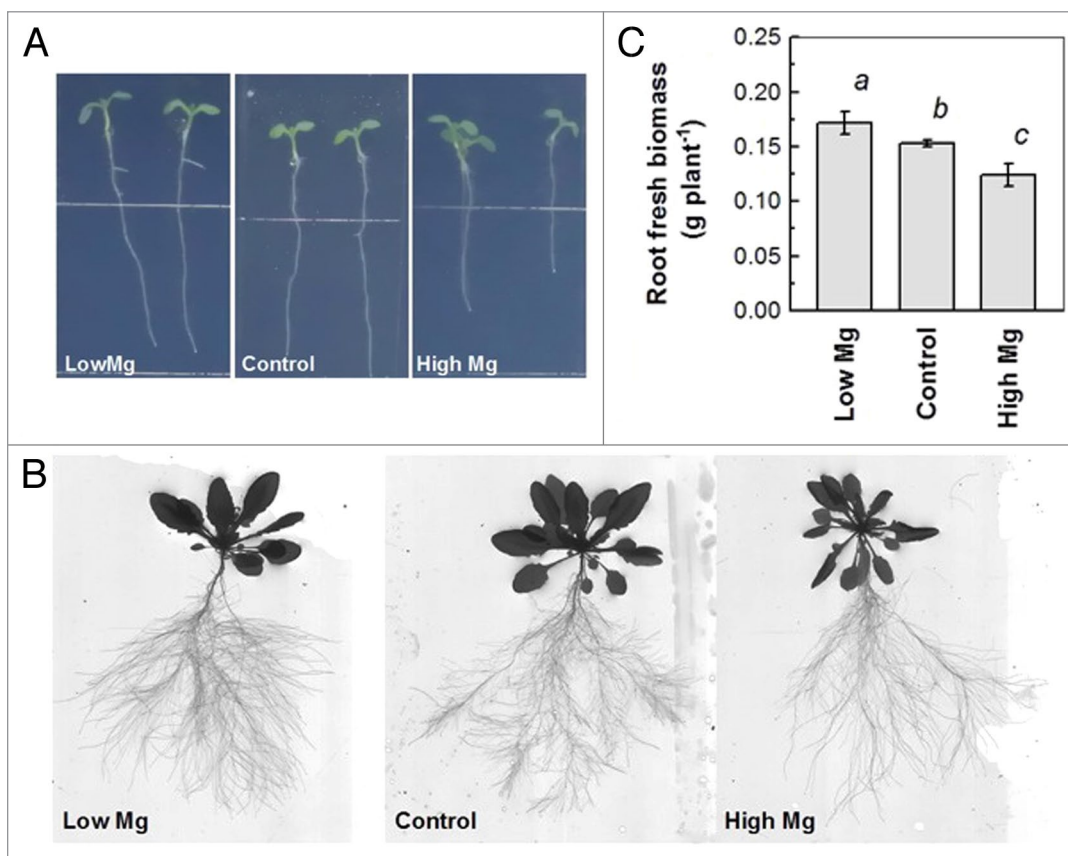
On the other hand, like other metals, Mg at high levels can deteriorate soil chemical and biological properties, and thus change the colonization and growth of plants. Large-scale magnesite mining activities drastically deteriorated the surrounding environment. In particular, Mg-rich dust derived from mining and calcination has led to intense vegetation and soil damage.<sup>7</sup> Hazards of excessive Mg intake to human health include changes in mental status, nausea, diarrhea, appetite loss, muscle weakness, breathing difficulty, extremely low blood pressure, and irregular heartbeat.<sup>8</sup> In the past decade, the importance of Mg in plant growth especially in plant development was underestimated.

Plants display an array of physiological responses to Mg availability, including morphological and architectural responses of the root system. The alteration of root architecture maybe a powerful green vehicle for the development of crop plants with an efficient Mg acquisition ability. Although evidence now exists for the influence of supply of most major- and micro-nutrients for the formation of roots,<sup>9-13</sup> there is relatively little attention has been paid on root growth in response to Mg excess compared with Mg deficiency. In recent years, the global transcriptional response to Mg imbalance has been studied by microarray technologies. For example, genome scale studies of the model species *Arabidopsis* under Mg depletion<sup>14,15</sup> or excess<sup>16</sup> have allowed unbiased views of the responses to Mg imbalance. The molecular targets among long-term (4-d and 7-d) under low Mg and high Mg by transcriptome sequencing technology (RNA-Seq) was also identified in our recent study.<sup>17</sup> These transcriptome profiling was conducted to detect genes whose expression could be significantly changed by Mg availability during root and root hair development. It opens up the opportunity of understanding on how plant root development in response to Mg availability and provides an alternative route of identifying genes responsible for sensing and signaling Mg nutrient limitations or excess. This report aims at: 1) evaluating the available knowledge on the influence of Mg nutrient on root growth and 2) highlighting insights into

\*Correspondence to: Yong Song Zhang; Email: yszhang@zju.edu.cn

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**Figure 1.** Magnesium (Mg) regulates root development in *Arabidopsis thaliana*. Light microscope images (A) of wild-type *Arabidopsis thaliana* seedlings grown in agar-medium supplied with 1  $\mu\text{M}$   $\text{MgSO}_4$  + 1000  $\mu\text{M}$   $\text{Na}_2\text{SO}_4$  (Low Mg), 1000  $\mu\text{M}$   $\text{MgSO}_4$  (Control), and 10000  $\mu\text{M}$   $\text{MgSO}_4$  (High Mg) for 7 d. Five-week-old wild-type *Arabidopsis thaliana* plants grown hydroponically were subjected to Mg treatment 1  $\mu\text{M}$   $\text{MgSO}_4$  + 1000  $\mu\text{M}$   $\text{Na}_2\text{SO}_4$  (Low Mg), 1000  $\mu\text{M}$   $\text{MgSO}_4$  (Control), and 10000  $\mu\text{M}$   $\text{MgSO}_4$  (High Mg) during a 1-wk period. At day 7 root morphology (B) and root fresh biomass (C) were taken and measured. Data are means  $\pm$  SD ( $n = 5$ ), and means followed by a same letter (*italics*) are not significantly different at  $P < 0.05$  in (C).

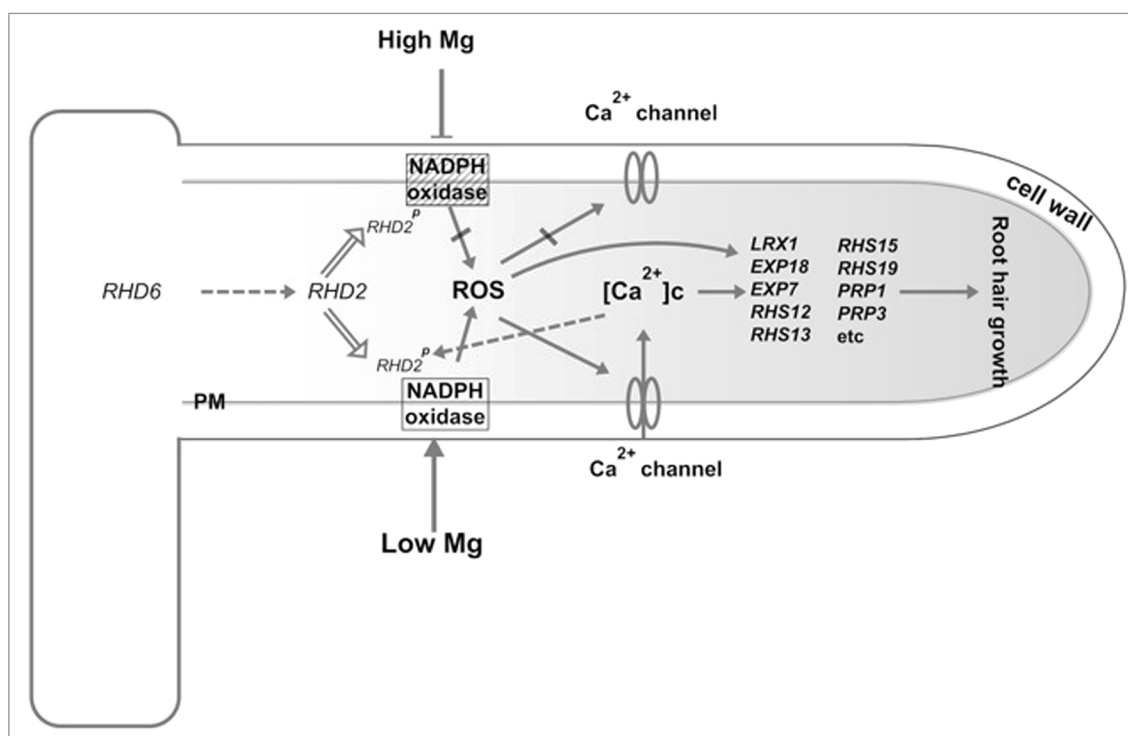
regulation of Mg availability on root and root hair development and its mechanism in *Arabidopsis* with respect to combining our published research of Niu et al. (2014).<sup>17</sup>

#### Root development response to Mg availability

In root cells, Mg is critical nutrient required for numerous fundamental biochemical processes, including energy generation, enzyme activation/inactivation, nucleic acid synthesis, nucleic acid folding, carbohydrate metabolism and the chemical catalysis of RNA splicing.<sup>18,19</sup> Therefore, impaired root absorption of Mg or root dysfunction from necrosis resulting in Mg deficiency will cause disruption of energy metabolism and biomass allocation. It has been reported that roots Mg concentration decreased quickly when removed Mg from the nutrient solution but maintained a constant low value,<sup>14</sup> indicating mobilization and redistribution mechanisms between organs. Previous researches reported that Mg deprivation (20 g/m<sup>3</sup>) for 12 d severely reduced the root biomass of bean (*Phaseolus vulgaris*) plants for accumulation of sugars and starch in leaves.<sup>20,21</sup> Damm et al. (2011)<sup>22</sup> suggested that low Mg availability together with high K supply enhanced the root-shoot ratio prior to any visible sign of Mg deficiency symptoms in rice leaves. This may be connected with the event that under Mg deficiency, carbon is more likely to be assigned

to the leaves instead of the root. In the present study five-week-old *Arabidopsis thaliana* (Col-0) plants grown hydroponically were supplying with Mg treatment 1  $\mu\text{M}$   $\text{MgSO}_4$  + 1000  $\mu\text{M}$   $\text{Na}_2\text{SO}_4$  (L), 1000  $\mu\text{M}$   $\text{MgSO}_4$  (Ctro), and 10000  $\mu\text{M}$   $\text{MgSO}_4$  (H) during the one-week period. At the end of the treatment, we showed that root fresh biomass decreased progressively with increasing Mg supply (Fig. 1). Furthermore, as compared with normal Mg, root-shoot ratio and total root surface area were greater in both low Mg and high Mg supplied plants but greater that of the low Mg-supplied plants (Data not shown). Further, it is suggested that root phenotype in *Arabidopsis* plants shown in Figure 1A and C solely resulting from Mg depletion and not from sulfur depletion (or both combined Mg and S depletion) in this study.

Recent excellent studies of Cristescu et al. (2013)<sup>23</sup> and Gruber et al. (2013)<sup>24</sup> have considered the systematic and exquisite strategies to study how plants adjust their root structure and morphology including total root length, primary root length, lateral roots number and length, first-order lateral roots (1° LR) and second-order lateral roots (2° LR) in response to Mg concentration. However, these publications do not specifically consider the effect of Mg supply on the root morphology with respects of root hair formation and elongation. Furthermore,



**Figure 2.** Schematic model showing the potential target points of Mg availability in the signaling events that lead to the growth of root-hair tips. Solid arrows indicate links established in the induction of root-hair development and broken arrows represent already established links in other systems but yet to be demonstrated in the growth of root-hair tips. Abbreviations: ROS, reactive oxygen species;  $RHD2^P$ ,  $RHD2$  with  $Ca^{2+}$ -dependent phosphorylation. NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase;  $cCa^{2+}$ , cytosolic  $Ca^{2+}$  concentration; PM, plasma membrane.

Gruber et al. (2013)<sup>24</sup> pointed out that although shoot growth was negatively affected by decreased S supply, the deficiency of S had relatively little influence on the morphology of roots. Therefore, in our present study, we mainly focus on analysis of primary root elongation and lateral root formation in response to Mg supply in the media. As shown in **Figure 1A**, primary root elongation and lateral root formation in *Arabidopsis* were not influenced by low Mg but inhibited by high Mg after one-week period (**Fig. 1**), suggesting a suppression of high Mg on root development. Therefore, root growth alternation may represent the earliest morphological response to low Mg conditions. This result is supported by the findings of other studies presenting that the absence of an effect on the root system of sugar beet,<sup>25</sup> *Arabidopsis*<sup>26</sup> and rice<sup>27</sup> grown hydroponically.

Hermans et al. (2010) identified early transcriptomic responses of *Arabidopsis* to Mg deficiency, and found that the highest number of regulated genes was observed first in the roots.<sup>15</sup> However, we cannot ignore the fact that the number of Mg deficiency responsive genes in leaves become gradually more important after 8 h and even after one week.<sup>14</sup> Fortunately, at least 2% genes in roots were changed in response to Mg limitation. Moreover, replenishment of Mg to the nutrient solution restored the initial patterns of gene expression for one-fifth of the transcripts in the leaves and half in the roots within 24 h,<sup>15</sup> suggesting that root development could be influenced by Mg concentration during this period. Verbruggen and Hermans (2013)<sup>28</sup> reported that none of the root parameters are

significantly different between Mg-deficient and Mg-adequate plants at the time of treatment using a hydroponics system. However, they pointed out that relative impact on the root or shoot growth depended on the plant species and the system. Besides, it is probable that there is a degree of difference between the effects of Mg depletion (without Mg added) and low Mg availability (micromolar Mg added on present study) on root development and genes expressions. Incidentally, Mg depletion did not alter the expression of most genes potentially involved in the transport and distribute of Mg ion.<sup>14,29</sup> These results were clearly different from reports on N, P and K inadequate, which have an definite impact on the root transcriptome at an early stage of induction, and eventually on root morphology.<sup>30-32</sup> However, few publications did specifically consider the mechanism strategies on how plants adjust their root structure and morphology in response to altered Mg conditions and the mechanism underlying this process deserve to be elucidated.

#### Root hair development under control of Mg availability

Our previous published study had first investigated the influence of Mg availability on root hair development in *Arabidopsis* grown in different Mg concentrations ranging from 0.5  $\mu$ M to 10 mM.<sup>17</sup> It is shown that the development of root hairs of *Arabidopsis* increased progressively with decreasing Mg supply after 7-d, which was related with the initiation of new trichoblast files and likelihood of trichoblasts to form hairs. Interestingly, the increasing concentration of Mg variation trend is consistent with the decrease of root hairs respond to

Mg availability. Moreover, the growth of root hairs of *mrs2-1mrs2-5* mutant affected in Mg transport and homeostasis was not altered by Mg deficiency but was tardily inhibited by high Mg, suggesting that internal Mg was partly related to the growth of root hairs but the mechanism underlying this process remains to be elucidated. Addition, increasing  $\text{MgSO}_4$  supply increased the concentration of total Mg and decreased the concentration of Ca in both roots and shoots irrespective of anion form supplied with Mg, such as sulfur or chloride. Surprisingly, there is no significant difference in S concentration in the roots in *Arabidopsis* grown in the medium containing  $\text{SO}_4^{2-}$  concentration ranging from 250 to 10250  $\mu\text{M}$ . This is maybe an alternative explanation on why sulfur has relatively little influence on the morphology of the root.

We predominately compared with previous root epidermis gene transcriptome data sets (Bruex et al. 2012)<sup>33</sup> and iRootHair database (a comprehensive root hair genomics database) from Kwasniewski et al. (2013).<sup>34</sup> Interesting outcomes of this global transcriptomics analysis are here identifying the differences in the expression of genes functioning in root hair or epidermis development under Mg treatments, which finally help us define a molecular mechanism of root hair development in response to Mg availability.<sup>17</sup> It is found a greater fraction of cell wall organization and morphogenetic H-genes genes were

down-regulated by high Mg but up-regulated by low Mg. Finally, a model is proposed on the target points of Mg in the signaling events that lead to the growth of root hair tips (Fig. 2). This simplified model is based on those proposed by Foreman et al. (2003)<sup>35</sup> and Takeda et al. (2008).<sup>36</sup> To sum up, it is possible that low Mg first increases while high Mg reduces ROS production through the activation (low Mg) or inhibition of NADPH oxidase activity.<sup>17</sup> Then ROS activate  $\text{Ca}^{2+}$ -channels at the plasma membrane and regulate cytosolic  $\text{Ca}^{2+}$  concentration in the apex of the root hair tip. These endogenous signals then modulate the downstream genetic elements of a number of genes and transcription factors (*e.g.* *LRX1*, *EXPI8*, *RHS12*, *RHS13*, *RHS15*, *RHS19*, *PRP1*, and *PRP3*) conferring cell wall organization and the growth of root hair tips.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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